gas into which they are projected. Langmuir suggested to the authors that if the mercury vapor stream were deflected and condensed at a definite point below the jet and inside the condenser, any observed volume change should be diminished. They are indebted to him for the suggestion of sealing an additional water-cooled tube into the condenser to accomplish this purpose, as shown by the heavy outlined portion of Figure 1. This modification definitely improved the reproducibility of calculated volumes at various pressures without impairing the pumping performance. Furthermore, the measured volume was now insensitive to mercury distillation rates.

The comparative results are shown in Figure 2. Curves I and II represent differences in calculated volumes as a function of gas pressure employing the conventional design of pump. Curve I is for the higher mercury distillation rate corresponding to 323 watts supplied to the electric resistance heater; and curve II, the lower distillation rate, corresponding to 240 watts. Curve III is for a similar system employing the modified diffusion pump. Determined volumes of the system are better reproducible and the maximum deviation of the mean volume as the pressure is lowered to 0.01 mm. of mercury is less than 10 ml. Of further importance is the fact that the data at three distillation rates practically coincide.

Literature Cited

(1) Chipman, J., and Fontana, M. G., IND. ENG. CHEM., ANAL. ED., 7, 391 (1935).
(2) Derge, G., *Metals Tech.*, 10, T. P. 1544 (1943).
(3) Holm, V. C. F., and Thompson, J. G., *Bur. Standards J. Research*, 26, 245 (1941).
(4) Langmuir, Irving, *Gen. Elec. Rev.*, 19, 1060 (1916).
(5) Vacher, H. C., and Jordan, L., *Bur. Standards J. Research*, 7, 375 (1931).

Modified Amino Nitrogen Apparatus for Insoluble Proteins

DAVID G. DOHERTY AND CLYDE L. OGG
Eastern Regional Research Laboratory, Philadelphia, Penna.

An auxiliary reaction chamber for use with a manometric Van Slyke apparatus is described. The apparatus enables one to determine easily the rate of liberation of amino nitrogen from insoluble proteins and thereby to determine their free amino nitrogen content.

SINCE the chemical modification of proteins frequently involves the free amino groups, it is advantageous to be able to determine the free amino nitrogen remaining after chemical treatment. Most of the protein derivatives are insoluble under the conditions of analysis, and some have a rather low free amino nitrogen content. The limitations of the Van Slyke method (*7*) are well known and have been summarized by Richardson (*5*). Its application to insoluble proteins has offered additional difficulties in the introduction of the sample into the reaction chamber and the selection of the time of reaction. Several means for solving these difficulties have been suggested (*1, 2, 3, 6*). Kanagy and Harris (*1*) have shown that an arbitrary reaction time may not give the correct value for free amino nitrogen in proteins and have utilized the rate of evolution of amino nitrogen to determine the free amino nitrogen present. Rutherford, Harris, and Smith (*6*) using a modified Shepherd gas analysis apparatus extended this method.

It seemed worth while to use this principle with an apparatus that was simpler and more adapted to routine determinations. With these requirements in mind an accessory reaction chamber for use with a standard manometric Van Slyke apparatus was designed which will permit introduction of large samples of solid material, removal of the gas evolved at stated intervals, and easy cleaning of the chamber at the end of the analysis. The calibrated Van Slyke extraction chamber is used only for measurement of the nitrogen evolved. The amino nitrogen content of several proteins and substituted proteins was determined with the aid of the auxiliary chamber.

Apparatus and Reagents

Figures 1 and 2 show the details of the auxiliary chamber and its relation to the Van Slyke apparatus. The chamber was constructed from a 34/45 standard taper ground-glass joint and mounted on a plate fastened to the extraction chamber carrier of the Van Slyke apparatus. The outer top half was constricted and sealed to a three-way 1-mm. bore capillary stopcock, *A*, fitted with a cup and a bent side arm similar to the stopcock of the Van Slyke extraction chamber. The inner bottom half was also constricted and sealed to a piece of 5-mm. bore heavy-walled tubing long enough to reach the pivotal point of the extraction chamber carrier. A yoke was fastened to each half of the chamber, and two connecting springs were provided to keep the chamber together. A 5-mm. bore stopcock, *B*, was fastened to the base of the Van Slyke apparatus and connected to the chamber with a piece of heavy-walled tubing. A 250-cc. leveling bulb was connected to *B* through a length of heavy-walled nitrometer tubing and hung in one of two rings on an adjacent ringstand. Sufficient mercury to fill the chamber and one third of the leveling bulb was added. The rings were arranged in such a manner that with the bulb in the lower ring the mercury level was slightly above the 5-mm. bore tube and with the bulb in the upper ring the level was 10 cm. above the top of the chamber.

The reagents used are a solution of 40 grams of sodium nitrite in 50 cc. of water, glacial acetic acid, a saturated solution of sodium acetate, and capryl alcohol. Rutherford, Harris, and Smith (*6*) have shown that addition of sodium acetate decreases the size of the blanks. The standard manometric Van Slyke-Hempel pipet is filled with a solution of alkaline permanganate consisting of 50 grams of potassium permanganate and 25 grams of potassium hydroxide made up to 1 liter with water. The ground-glass joint of the chamber is well lubricated with a stiff vacuum grease before being assembled. The samples used are all ground to 80-mesh in a Wiley micromill, and the moisture content is determined at the time of analysis. The amount of protein used ranges from 0.5 to 1 gram.

Experimental Procedure

An excellent description of the care and use of the manometric Van Slyke apparatus has been given by Peters and Van Slyke (*4*). One familiar with the standard manometric amino nitrogen determination may proceed with the modified method as follows:

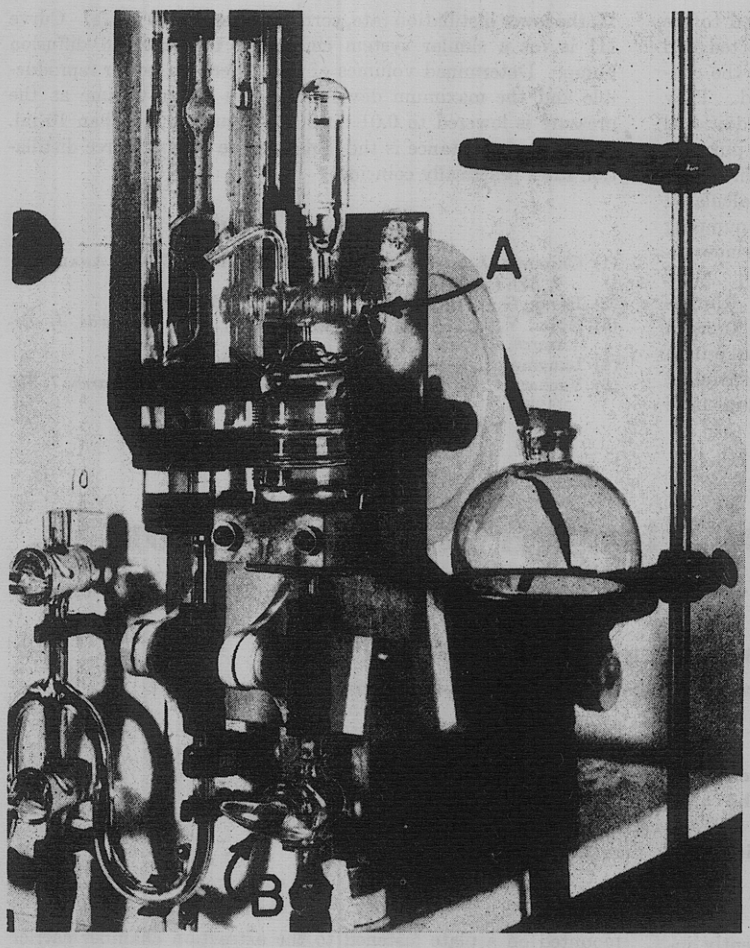

FIGURE 1. REACTION CHAMBER ATTACHED TO VAN SLYKE APPARATUS

Ten milliliters of water are admitted to the calibrated Van Slyke extraction chamber, the stopcock is closed, and the mercury is set at the 50-ml. mark. The top of the auxiliary chamber is removed, the mercury level set even with the bottom of the chamber, and stopcock *B* closed. The weighed sample of solid protein is introduced, followed by 2.0 ml. of glacial acetic acid, 2.0 ml. of saturated sodium acetate solution, and 5 drops of capryl alcohol. The chamber is assembled, and the bore of stopcock *A* and the capillary of the cup are filled with water. The side arm of *A* is connected with a high-vacuum pump, the pump started, and *A* turned to connect the pump with the chamber. The evacuation is continued for 2 minutes. The chamber is shaken throughout this period to facilitate removal of air from the mixture and prevent the formation of a cake. The shaking is then stopped, *A* is closed, and the vacuum line is disconnected.

Four milliliters of sodium nitrite solution are pipetted into the cup and run into the chamber by turning *A*, care being taken to admit no air. The cup and the bore of the stopcock are rinsed with 1 ml. of water, and the chamber is shaken 5 to 15 minutes, depending upon the amount of gas evolved. The shaking is then discontinued and *B* is opened to admit mercury to the chamber at an even rate. The leveling bulb is raised to the high ring to place the gas in the chamber under positive pressure. A few milliliters of water are placed in the cup, and the tip of a Hempel pipet is pressed in place. The Hempel stopcock is turned to connect the tip with the bulb, *B* is closed, and *A* is opened wide. *B* is then slowly opened, admitting mercury to the chamber and forcing the evolved gas into the Hempel pipet. *B* is closed when the reaction mixture is even with the bore of *A*. The Hempel stopcock is turned to the off position, the leveling bulb placed in the low ring, and *B* opened and then closed. The Hempel stopcock is turned to connect the cup with the tip, and *B* is opened slowly, allowing the gas in the Hempel capillary to be drawn back into the chamber along with enough water to seal the bore of *A*. *A* is closed, and the mercury level in the chamber is lowered until it is even with the bottom. *B* is then closed. The purified nitrogen in the Hempel pipet is transferred to the calibrated extraction chamber and measured in the usual manner.

Successive samples of gas are removed by the same procedure, the chamber being shaken for 10 minutes just prior to removal. The most effective control of liquids and gases is obtained through manipulation of the flow of the mercury through *B*.

Blank determinations were run in a similar manner, the protein being omitted. Selected time intervals generally were 10 minutes, 30 minutes, 1 hour, and each succeeding hour, the time being taken from the moment the sodium nitrite was added to the mixture. If the protein clings to the walls of the chamber when the mercury is lowered after a sample of gas has been removed, it may be dislodged by shaking the chamber while the mercury level is being lowered. The pressure readings obtained were corrected by subtracting a blank value for a similar time interval. The rate curves were obtained by plotting the total amino nitrogen liberated, in milligrams per gram of dry protein, against time. Duplicate values obtained from several proteins, modified proteins, arginine, and α-benzoylarginine amide by extrapolation of the secondary portion of the curve to zero time are given in Table I.

Discussion

Determinations were run in duplicate, and the rate curves for proteins and derivatives (Figure 3) plotted as previously described. The ratio of amino nitrogen to total nitrogen was plotted against time for arginine and α-benzoylarginine amide (Figure 4). The determinations were reproducible within the same limits of error as the manometric Van Slyke amino nitrogen determination (*4*). The values for the free amino nitrogen content of the proteins and derivatives in Table I were found by extrapolation of the secondary portion of the rate curve to zero time. This extrapolation is illustrated by the gelatin curve in Figure 3. The values can also be determined by calculation, using the straight-line equation:

$$y = mx + b$$

TABLE I. FREE AMINO NITROGEN CONTENT OF SOME PROTEINS AND DERIVATIVES DETERMINED AT 22–23° C.

Substance	Amino Nitrogen (Duplicate Determinations)	
	Mg./g. dry substance	
Wool	3.30	3.35
Silk (raw)	2.90	2.96
Gelatin (Eastman Kodak Co.)	8.70	8.74
Zein (Corn Products Refining Co.)	1.00	...
Casein	8.40	8.35
Benzoyl casein[a]	0.20	0.18
Desamino casein[a]	0.90	1.00
Arginine	80.2[b]	80.2
α-Benzoylarginine amide	0.0	0.0

[a] Prepared by J. L. Wood and A. P. Swain of this laboratory.
[b] Calculated, 80.4.

where y is the total milligrams of nitrogen per gram of dry protein obtained in time x, m the milligrams of nitrogen obtained per hour when the rate has become constant, and b the amount of free amino nitrogen initially present.

To achieve reproducible results and a rate curve capable of analysis by these methods, it is important to prevent the formation of aggregates in the chamber when the reagents are added. The material in the lumps so formed is not readily accessible to the action of the reagents and consequently evolves nitrogen slowly. The rate curve plotted from the values obtained in this instance has no definite change in slope and cannot be extrapolated in the usual manner to obtain an amino nitrogen value. This factor of aggregation is also of importance when the solution of a protein which is precipitated under the conditions of the analysis is admitted into the chamber. Thus the curd formed by an alkaline casein solution gave an indecisive rate curve similar to those obtained when aggregates of solid material were present. The use of a finely divided casein, well shaken to prevent the formation of lumps, obviated this difficulty. An examination of the curves obtained for the various proteins shows that one cannot arbitrarily select any reaction time that will be valid for different substances and conditions of analysis. Once the technique of handling this modified apparatus is acquired, the time required for the removal and measurement of a sample of gas—about 5 minutes—is approximately the same as in the standard Van Slyke determination. Since the reaction chamber is easily accessible and can be taken apart, it can be quickly and thoroughly cleaned at the end of each determination.

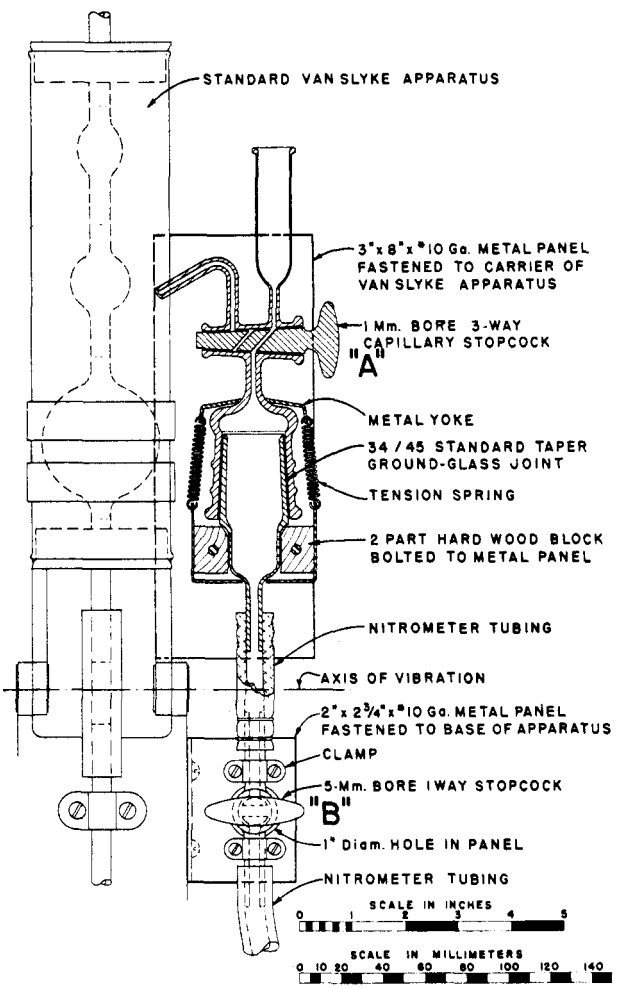

FIGURE 2. DIAGRAM OF REACTION CHAMBER

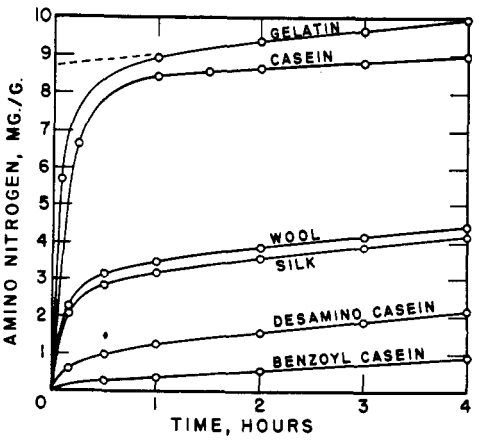

FIGURE 3. RATES OF EVOLUTION OF NITROGEN FROM VARIOUS PROTEINS

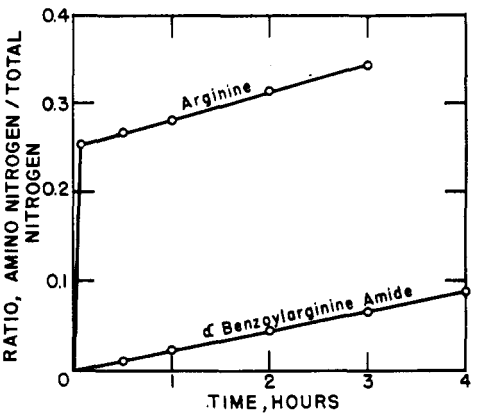

FIGURE 4. RATES OF EVOLUTION OF NITROGEN FROM ARGININE AND α-BENZOYLARGININE AMIDE

Summary

A simple apparatus for the routine determination of amino nitrogen of insoluble proteins and protein derivatives has been described. The rate of evolution of amino nitrogen from some insoluble proteins and protein derivatives was determined with this apparatus. The curves obtained by plotting the amino nitrogen content against time are similar to those obtained by Rutherford, Harris, and Smith (6) using a modified Shepherd gas analysis apparatus.

Literature Cited

(1) Kanagy, J. R., and Harris, M., *J. Research Natl. Bur. Standards*, 14, 563 (1935); *Research Paper* 787.
(2) Klemm, O., and Meyer, K. H., *Helv. Chim. Acta*, 23, 1444 (1940).
(3) Lieben, F., and Loo, Y. C., *J. Biol. Chem.*, 145, 223 (1942).
(4) Peters, J. P., and Van Slyke, D. D., "Quantitative Clinical Chemistry", Vol. II (Methods), p. 385, Baltimore, Williams & Wilkins Co., 1932.
(5) Richardson, G. M., *Proc. Roy. Soc.*, Ser. B115, 142 (1934).
(6) Rutherford, H. A., Harris, M., and Smith, A. L., *J. Research Natl. Bur. Standards*, 19, 467 (1937); *Research Paper* 1038.
(7) Van Slyke, D. D., *J. Biol. Chem.*, 9, 185 (1911).